Multiple Forms of Lysyl-Transfer Ribonucleic Acid Synthetase in *Escherichia coli*

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Lysyl-transfer ribonucleic acid synthetase (EC 6.1.1.6) was identified as four polypeptide spots after two-dimensional polyacrylamide gel electrophoresis of whole-cell lysates of *Escherichia coli*. Identification was made by migration with partially purified enzyme preparations, by peptide map patterns, by mutant analysis, and by correlation of spot intensities with changes in enzyme levels under different growth conditions. Wild-type cells growing at 37°C in glucose minimal medium displayed the enzyme predominantly as two spots (spots I and III). Growth at 46°C, growth in the presence of alanine or glycyl-L-leucine, or growth of a strain with a mutational deficiency in S-adenosylmethionine synthetase (*metK*) greatly increased the synthesis of two other spots (spots II and IV). Polypeptides I and III, but not polypeptides II and IV, had altered isoelectric points in a lysyl-transfer ribonucleic acid synthetase mutant. These data suggest that multiple forms of lysyl-transfer ribonucleic acid synthetase exist in vivo and that they may be encoded by more than one gene.

Aminoacyl-tRNA synthetases are subject to multiple modes of regulation in *Escherichia coli*. The deprivation of an amino acid results in either a long-term or a transient increase in its cognate synthetase (16, 18, 19, 22, 28). The levels of the synthetases are also a function of the cellular growth rate (20, 22, 24). A third situation has been reported for lysyl-tRNA synthetase (10). The activity of this enzyme can be influenced by the presence of alanine (9, 11, 13, 14) or leucine dipeptides, such as glycyl-L-leucine (3, 11, 13), and by a mutation in an apparently unrelated gene, the structural gene for S-adenosylmethionine synthetase (10).

We report here that lysyl-tRNA synthetase from cells grown in minimal medium appeared as two polypeptides, which differed in isoelectric point. Conditions that increased the activity of this synthetase promoted the synthesis of two new polypeptide species with higher apparent molecular weights but similar isoelectric points. The various forms appeared to exist in vivo (possibly encoded by more than one gene), indicating that lysyl-tRNA synthetase regulation is unusual.


**MATERIALS AND METHODS**

**Bacterial strains.** The *E. coli* strains used in this study are listed in Table 1.

**Media.** In all experiments cells were grown in MOPS minimal medium (21) or rich medium (20); 0.4% (wt/vol) glucose was used as the carbon source. When glycyl-L-leucine was present in the medium, it was necessary to include isoleucine (0.3 to 0.4 mM) in order to prevent growth inhibition by the peptide (27). Isoleucine was also present in the control culture. Supplements for strains IH2000 and IH2001 were lysine and threonine (0.4 mM), cysteine (0.2 mM), leucine (0.5 mM), and vitamin B1 (0.01 mM).

**Bacterial growth.** All cultures were grown aerobically on a rotary shaker at 37 ± 0.1°C. The growth of the cells was monitored at 420 nm, and the cells were grown to an optical density of 1.0 (approximately 10⁸ cells per ml). The growth of radioactive cultures was followed by monitoring parallel cultures of unlabeled cells.

**Synthetase assay.** Lysyl-tRNA synthetase activity was assayed in crude extracts as described by Hirshfield et al. (10, 14). Protein was determined by the method of Lowry et al. (17), using crystalline bovine serum albumin as a standard.

**Determination of steady-state polypeptide levels.** Steady-state cultures of the strains were grown in MOPS glucose minimal medium containing either L-[³H]leucine (115 mCi/mmole; 125 μCi/ml) or L-[¹⁴C]lysine (1 Ci/mmole; 75 μCi/ml) in the reference cultures. The experimental cultures contained L-[¹⁴C]leucine (300 mCi/mmole; 50 μCi/ml) or L-[¹⁴C]lysine (270
leucine in the experiments with the metK strain, and lysine was used in the other experiments. The concentration of leucine in the medium was 0.16 mM, and the concentration of lysine 0.09 mM.

Extracts were prepared as described by Blumenthal et al. (1). Portions (20 μl) were analyzed by using the two-dimensional system of O’Farrell (23). The isotopes were counted by sample oxidation, as described by Neidhardt et al. (20). The [1-14C]H ratio for each protein divided by the [14C]/H ratio for the total mixture (the unresolved extract) gave the level of that protein in the experimental culture compared with the reference culture.

Table 1. E. coli K-12 strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG (wild type)</td>
<td>metK*</td>
<td>R. Greene</td>
</tr>
<tr>
<td>RG62</td>
<td>metK</td>
<td>R. Greene</td>
</tr>
<tr>
<td>IH2000</td>
<td>thi lys cya thy</td>
<td>I. Hirshfield</td>
</tr>
<tr>
<td></td>
<td>leu lysS*</td>
<td>I. Hirshfield</td>
</tr>
<tr>
<td>IH2001</td>
<td>thi lys cya thy</td>
<td>I. Hirshfield</td>
</tr>
<tr>
<td></td>
<td>leu lysS</td>
<td>I. Hirshfield</td>
</tr>
<tr>
<td>NC3</td>
<td>Wild type</td>
<td>F. C. Neidhardt</td>
</tr>
</tbody>
</table>

mCi/mmol; 100 μCi/ml). Leucine was used in the experiments with the metK strain, and lysine was used in the other experiments. The concentration of leucine in the medium was 0.16 mM, and the concentration of lysine 0.09 mM.

Extracts were prepared as described by Blumenthal et al. (1). Portions (20 μl) were resolved on a two-dimensional gel (20). The isotopes were counted by sample oxidation, as described by Neidhardt et al. (20). The 14C/H ratio for each protein divided by the 14C/H ratio for the total mixture (the unresolved extract) gave the level of that protein in the experimental culture compared with the reference culture.

Determination of the number of molecules of lysyl-tRNA synthetase per genome. Strain RG62 (metK) was grown with 11-14Cglucose (504 mCi/mmol; Amersham); the medium contained 9.4 mM radioactive glucose (27 μCi/mmol). Cell extracts were prepared, two-dimensional gels were run, and entire protein spots were punched out to count total radioactivity.

The total radioactivity in each synthetase form was measured and expressed relative to the total radioactivity of protein chain elongation factor G. The calculation of α, the fraction of the total protein represented by each protein, was based on the assumption that under the conditions of growth of strain RG62 in glucose minimal medium, one EF-G molecule was present per 70S ribosome (7) and on the assumption that the fraction of total protein which was represented by the ribosomal proteins was 0.137 (5). This gives an α value for EF-G of 0.0166 (26). We also assumed that the recovery of forms I through IV from the gels was equivalent to the recovery of EF-G (25). Thus, the α for each lysyl-tRNA synthetase form was calculated from the following expression: [(counts per minute of lysyl-tRNA synthetase form)/counts per minute of EF-G] × 0.0166.

The number of molecules of each lysyl-tRNA synthetase form per genome equivalent of DNA was derived by multiplying the amount of protein per genome equivalent of DNA (4 × 109 amino acid residues × 110 daltons per average amino acid, or 4.4 × 1010 daltons [5]) times the α value and then dividing this number by the molecular weight of the polypeptide. For forms I and III 58,500 was used as the molecular weight, and for forms II and IV 60,500 was used.

The determination of the number of molecules of each synthetase form in the metK strain permitted the calculation of the number of molecules of each form in other strains grown in glucose minimal medium with or without alanine and glycyl-L-leucine. This relationship was derived from the determination of the relative amount of each form, as described above. From this information the relationship between the metK strain and the wild-type strain was determined, and thus it was possible to calculate the number of molecules of each form in the wild-type strain in the absence of dipeptide or L-alanine.

Peptide mapping. Cells (0.5 mg, dry weight; 5 units of absorbance at 420 nm) were labeled with 10 μM 35SO4 (108 μCi/mmol) in glucose minimal medium containing 3 mM glycyl-L-leucine. Each of the four forms of the synthetase was removed from a two-dimensional gel, digested with Staphylococcus aureus protease V8 (0.25 μg), and electrophoresed on a 16% sodium dodecyl sulfate–polyacrylamide gel as described by Cleveland et al. (4). Autoradiograms of the gels were developed by using the procedure of Bonner and Laskey (2).

RESULTS

Forms of lysyl-tRNA synthetase observed on two-dimensional gels. Figure 1 shows an autoradiogram of an O’Farrell gel made from strain IH2000 wild-type cells grown on glucose minimal medium at 37°C. The spot directly above the Roman numeral I was originally identified (20) as lysyl-tRNA synthetase by its migration with a purified sample of this enzyme (13). Immediately to the right of spot I is a fainter spot labeled III, which we will show is also lysyl-tRNA synthetase. No spots are visible in this autoradiogram at the locations marked by circles at II and IV. When this strain was grown in the presence of 20 mM L-alanine (9, 12, 14) or 3 mM glycyl-L-leucine (3, 10) (con-
ditions known to elevate lysyl-tRNA synthetase activity in E. coli), polypeptide spots appeared at locations II and IV. This is illustrated for L-alanine by the autoradiogram in Fig. 2A. Mutant strain RG62 (metK), which has an elevated synthetase activity (10), displayed all four spots, even when cultivated in unsupplemented minimal medium (Fig. 2B). Wild-type strains cultivated at 46°C also displayed all four spots (data not shown).

Biochemical identification of spots II through IV. Identification of spots II through IV as lysyl-tRNA synthetase species was confirmed by two biochemical tests. First, enzyme was prepared from strain IH2000 (grown with 3 mM glycyl-L-leucine) to 50% purity through a Biorex 70 fraction as described by Hirshfield et al. (13) and then was examined by using a two-dimensional gel. All four synthetase forms were present (data not shown). A more extensive analysis of the putative lysyl-tRNA synthetase species was performed by peptide mapping each spot cut from a two-dimensional gel of strain IH2000 grown with glycyl-L-leucine. These peptides were compared with peptides obtained from form I lysyl-tRNA synthetase. Figure 3 shows that forms I and III had identical sets of bands. The bands of form III were lighter because less of this form was present. The peptides of form II were similar to the peptides of form I, with two exceptions. Band A appeared to be deficient in form II compared with form I or III. This area of the gel was faint, but more of peptide A was visible in form III than in form II, even though form II was present in a higher amount on the two-dimensional gel. The second exception (band B) was the extra band present in form II. Form IV migrated adjacent to an unknown spot on the O'Farrell gel and therefore was difficult to remove without contamination from the unknown protein. The peptide map of form IV showed many bands similar to the bands of the other forms, but also several new bands, which presumably were due to a contaminant.

The similarity of the peptide maps of forms I and III suggests that these two polypeptides may differ only by a modification. Despite their positional differences, forms I through III (and probably form IV) must have similar primary structures in order to yield peptide maps with so many bands in common.

Analysis of a lysyl-tRNA synthetase mutant. Strain IH2001 has only 5% of the lysyl-tRNA synthetase activity found in strain IH2000 (14). An analysis of this mutant grown on unsupplemented minimal medium revealed that the forms visible in the wild-type strain under these conditions were identical to those of wild-type E. coli.
conditions (forms I and III) had apparently shifted to the left (basic direction). An alternative possibility (that form I was unchanged and form III moved to the left of form I) cannot be ruled out at this time, but is less likely on the basis of the relative amounts of the two forms. Growth in the presence of L-alanine induced the appearance of forms II and IV in their normal locations (Fig. 4). The polypeptide map patterns of forms II and IV from the mutant strain were indistinguishable from the patterns of the analogous forms from wild-type cells, confirming the shift in the isoelectric positions of spots I and III. This finding provides further support for the identification of spots I and III as lysyl-tRNA synthetase and suggests that a second gene (or more) may be involved in the production of spots II and IV.

Amounts of the various forms. Cultures of wild-type strain IH2000 were prepared in glucose minimal medium containing [3H]lysine, and these cultures served as reference cultures; cells from these cultures were mixed with [14C]-labeled cells of the same strain grown in medium containing 3 mM glycyl-L-leucine or 20 mM L-alanine. The reference cells were also mixed with cells of the metK mutant strain RG62 grown in minimal medium. By using gels prepared from these mixtures we measured the amounts of spots I through IV relative to their amounts in the reference culture. The molecular amount of each was then calculated, based on the total amount of label in each spot of a reference culture, as explained above (Table 2).

The absolute levels of the spots in mutant strain IH2001 were not measured directly, but they were similar to the levels in the parental strain grown in minimal medium, based on a visual inspection of the gels. The relative increases in the spots in the mutant after growth with glycyl-L-leucine were measured in the usual way, by mixing a [3H]-labeled reference culture (IH2001 in minimal medium) with a [14C]-labeled experimental culture (IH2001 in medium containing glycyl-L-leucine) (Table 3).

Taken together, the results in Tables 2 and 3 indicate that total lysyl-tRNA synthetase activity was proportional to the molar sum of the activities of all four species in the strains (IH2000 and RG62) with normal enzyme and that growth with the dipeptide or L-alanine brought about normal increases in forms II and IV in the mutant strain. These results suggest the following simple description of the mutant phenotype and

![Figure 4](http://jb.asm.org/)

**Fig. 4.** Portion of an autoradiogram of a two-dimensional gel of mutant IH2001 grown in glucose minimal medium containing 20 mM L-alanine. The lysyl-tRNA synthetase forms are labeled as described in the legend to Fig. 1.

### Table 2. Molecular amounts of lysyl-tRNA synthetase species

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth medium supplement*</th>
<th>Form I</th>
<th>Form II</th>
<th>Form III</th>
<th>Form IV</th>
<th>Relative enzyme sp act*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IH2000</td>
<td>Glycyl-L-leucine</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>IH2000</td>
<td>t-Alanine</td>
<td>1.4</td>
<td>11.5</td>
<td>1.2</td>
<td>2.1</td>
<td>0.70</td>
</tr>
</tbody>
</table>

*The growth medium was glucose minimal medium, except in those cases where it was supplemented with 3 mM glycyl-L-leucine or 20 mM L-alanine, as indicated.

*Enzyme activity (units per milligram of protein) is expressed relative to the amount of activity in the wild-type strain grown in minimal medium.

*Enzyme activity was not measured in the same experiment as the molecular levels; a two- to fourfold increase was observed in other experiments.

### Table 3. Effects of growth supplements on molecular species of lysyl-tRNA synthetase in a lysS mutant

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth medium supplement*</th>
<th>Relative amt*</th>
<th>Relative enzyme sp act*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Form I</td>
<td>Form II</td>
<td>Form III</td>
</tr>
<tr>
<td>IH2000</td>
<td>Glycyl-L-leucine</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>t-Alanine</td>
<td>1.4</td>
<td>11.5</td>
</tr>
</tbody>
</table>

*The growth medium either was glucose minimal medium or was supplemented with 3 mM glycyl-L-leucine, as indicated.

*The level of each form in the culture supplemented with the dipeptide was measured relative to the level in the same strain grown in minimal medium lacking the dipeptide.

*The enzyme activity (units per milligram of protein) is expressed relative to the activity in wild-type strain IH2000 grown in minimal medium.
of the regulation of lysyl-tRNA synthetase formation. Forms I and III were active in the wild-type strain, but were largely inactive in the mutant (IH2001) as a result of the mutation that changed isoelectric points of these forms. Growth with glycyl-L-leucine or L-alanine induced another lysyl-tRNA synthetase (forms II and IV), which was responsible for the increased enzyme activity observed in all strains.

**Origin of induced form II.** We performed an experiment to determine whether the addition of glycyl-L-leucine induced de novo synthesis of form II or simply brought about a conversion of form I to form II. Cells of wild-type strain NC3 were grown in glucose minimal medium containing [14C]leucine. After a chase with non-radioactive leucine, the cells were washed and allowed to grow for 1 mass doubling in medium supplemented with glycyl-L-leucine. A parallel culture was treated identically, except that [14C]leucine was present only during the cell mass doubling in the presence of glycyl-L-leucine. Therefore, these two cell populations had the same high content of both forms I and II, but gels prepared from them revealed that induction involved de novo synthesis of form II; this form was derived neither from form I nor from any other preexisting protein in the cells.

**DISCUSSION**

In previous work it was discovered that diverse substances, such as alanine and glycyl-L-leucine, which are not substrates of lysyl-tRNA synthetase, enhance the cellular level of activity of this enzyme (3, 13, 14). In addition, a metK mutation that reduces S-adenosylmethionine synthetase activity to very low levels (8) also increases the activity of lysyl-tRNA synthetase (10). These effects are not additive; glycyl-L-leucine does not increase enzyme activity in a metK strain (10). The present study shows that each of these conditions promotes the synthesis of two polypeptides that are expressed at only very low levels in wild-type _E. coli_ cells growing in glucose minimal medium. The increased synthesis of these polypeptides (designated forms II and IV) occurs without diminution of the levels of the two polypeptides (forms I and III) found in normal cells growing in minimal medium. The same process takes place in both _E. coli_ B strains and _E. coli_ K-12 (data not shown).

We believe that all four of these polypeptides are lysyl-tRNA synthetase because all exhibit similar peptide patterns, because all are present in purified synthetase preparations from appropriate cultures, and because variations in their levels in mutant and wild-type strains correlate with total lysyl-tRNA synthetase activity. Based on results with the lysyl-tRNA synthetase mutant strain IH2001, it appears that at least two of the forms (forms I and III), are encoded by the mutant gene, whereas the other two forms (forms II and IV) are products of a second gene or set of genes that normally are cryptic during growth in minimal medium.

The fact that two forms are altered in the lysyl-tRNA synthetase mutant and the fact that two other forms are induced by certain growth conditions raise the suspicion that each pair might consist of a single polypeptide plus a modified version of this polypeptide. There are many examples of bacterial proteins that exist in multiple forms; for example, the N-terminal modification of ribosomal protein S6 generates locational changes on gels (26) similar to those observed with lysyl-tRNA synthetase forms I and III or forms II and IV. Confirmation of this hypothesis awaits chemical studies on the separate polypeptides and the analysis of more mutants.

The induced species form II is not derived by degradation or processing of form I. The addition of glycyl-L-leucine induces a 10-fold increase in the de novo synthesis of form II (Table 4). Furthermore, pulse-labeling a culture at 46°C revealed that neither form I nor II can be a precursor of the other (data not shown).

The existence of two genes for the same protein also has precedent; the products of the two genes for elongation factor Tu (15) differ by only one peptide (6). Our results indicate that there may be two or more genes for lysyl-tRNA synthetase, but a conclusive statement cannot be made until the genes can be identified by appropriate mutants, can be mapped, and can be assigned their respective products.

The circumstances that induce functioning of the putative second gene are puzzling. It is not

**Table 4. De novo synthesis of lysyl-tRNA synthetase form II after induction of _E. coli_ NC3 by glycyl-L-leucine**

<table>
<thead>
<tr>
<th>Labeling of cells</th>
<th>Form I</th>
<th>Form II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before glycyl-L-leucine addition</td>
<td>265</td>
<td>24</td>
</tr>
<tr>
<td>In the presence of glycyl-L-leucine</td>
<td>315</td>
<td>323</td>
</tr>
</tbody>
</table>

*a* Radioactivity in entire spot normalized between gels to 1,000 cpm in spot B40.7 (α subunit of RNA polymerase).
clear what common physiological signal might be generated by growth with L-alanine or glycyll-leucine, growth with a deficiency in S-adenosylmethionine synthetase activity, and growth at a high temperature. The results at high temperature were obtained in a rich medium containing all amino acids, nucleotide bases, and vitamins, so a temperature-sensitive defect in methionine or isoleucine biosynthesis cannot be invoked to explain the observed induction at 46°C. Whatever the outcome of further analysis of this induction, it is clear that the regulation of lysyl-tRNA synthetase is unusual and that it must be studied by measuring individual polypeptides rather than enzyme activity in whole-cell extracts.

Of the 20 aminoacyl-tRNA synthetases, 17 have been identified on standard two-dimensional gels, and only lysyl-tRNA synthetase shows visible evidence of existing in multiple forms or of varying in this manner with alanine or dipeptide supplementation, high temperature, or the metK restriction. Nevertheless, we are not certain that this synthetase is unique; others may have escaped notice by being located in congested areas or by having forms that are located at positions remote from the primary form. Computer-assisted scanning of autoradiograms should help answer whether the enzyme for lysine has a unique form of regulation.

ACKNOWLEDGMENTS

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LITERATURE CITED
